INFLUENCE OF STRONG STATIC MAGNETIC FIELD ON BIOELECTRICAL CHARACTERISTICS OF RAT HEMIDIAPHRAGM MUSCLE

MUSTAFA ITEGIN*
ISMAIL GÜNAY*

SUMMARY: The bioelectrical changes in the skeletal muscle and nerve-muscle junction of rats exposed to static magnetic field of 200 Gauss for a long periods were investigated in in-vitro conditions. All records were taken intracellularly, by microelectrode technique. It was demonstrated that chronic magnetic field exposure leads to depolarization in the skeletal muscle fiber membrane, and also causes the amplitudes of muscle action potentials and miniature end-plate potentials to decrease significantly. It was also determined that magnetic field leads to similar effects with ketamine, and that both potentiates the effects of each other.

Key Words: Magnetic fields, neurophysiology, neural transmission, ketamine.

INTRODUCTION

The effects of magnetic fields on cellular function are inconclusive and often generate conflicting results (1-3). Although there now exists a substantial literature dealing with the possible biological effects of exposure to electromagnetic and magnetic fields, the results are still often considered to be contentious (2,3). More recently substantial evidence have been presented indicating that strong static magnetic fields can influence biological systems. This is especially true about the influence of these fields on the excitable cells such as muscle and nerve fibers (4-7).

The present study investigated the in vitro effects of the static magnetic field on the rat phrenic nerve - hemidiaphragm preparation.

MATERIALS AND METHODS

Subjects

The initial weights of the weaning Wistar rats (n=20) that we used in our experiments ranged between 35 to 43 g. These rats were exposed to magnetic field for nineteen weeks. The rats were separated to two groups (n=10 each) and kept in two separate stainless steel cages; one of the groups served as the control group.

The distance between the experimental and control cages was two meters. All animals were fed and watered ad libitum, and kept in an environmentally controlled room at 21-23°C, with a light/dark schedule of 12/12 hours.

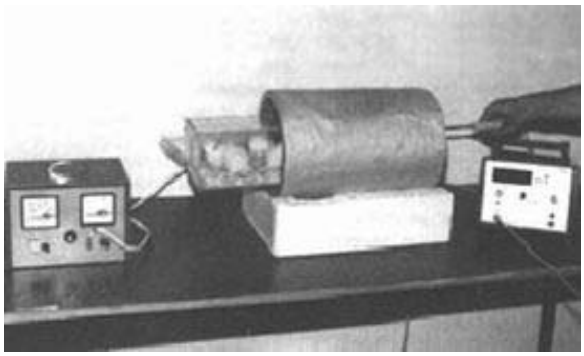
Application of static magnetic field

The magnetic field was generated in a specially designed device which had a solenoid 400 mm in length and with a diameter of 210 mm. The maximum value of induction in the center of the solenoid was 100 mT. This magnet was constructed by winding 1400 turns of 1.4 mm diameter insulated soft copper wire on a fiber roof. A DC current, produced by a DC current source is applied through it. The investigations presented here were performed in a horizontal magnetic field with an induction of B=200 G. Magnetic field intensity was measured by a digital Teslameter (Phywe), with an axial Hall-effect probe.

Inside the solenoid and between the poles of the electromagnet, a cage (40x17x13 cm) that was made of plexiglass

* From Department of Physiology, Faculty of Medicine, Çukurova University, Türkiye.

Figure 1: Demonstration of magnetic field exposure.



was placed in the homogeneous magnetic field. Six experimental animals were placed in this cage, in turn in order to expose them to magnetic field.

The animals were exposed to magnetic field for four hours a day for 19 weeks.

The solenoid was always kept in north-south direction, and its temperature was kept constant at 37°C.

Phrenic nerve-hemidiaphragm preparation and bioelectrical recordings

Resting membrane potential (RMP), action potential (AP) and miniature end-plate potentials (MEPPs) were recorded from the rat phrenic nerve-hemidiaphragm preparation. The animals used for these experiments were Wistar rats and their weights ranged from 200 and 230 grams. The rats without any previous preparation were sacrificed by decapitation and their rib cages were removed and trimmed around the diaphragm (8). The left hemidiaphragm of the decapitated Wistar rats was excised and preparation was preserved in a 10 ml plexiglass chamber at the base of perfused with Krebbs solution (in mM/L : 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 0.6 $MgSO_4$, 1.17 KH_2PO_4, 25 $NaHCO_3$, 11.1 glucose). Krebbs solution was supplied with a mixture of 95% O_2 and 5 % CO_2. The pH of the perfusate was kept constant at 7.4, and the rate of perfusion was 2 ml per minute. The temperature was maintained between 35.5 and 36.5°C (± 0.5).

RMP, AP and MEPPs of the rats diaphragm muscle were recorded intracellularly using conventional glass capillary electrodes (0.1mm O. D. and 10 to 30 MΩ resistance) filled with 3 M KCl, of specific concentrations (30, 50, 70, 90, 100 and 120 μM). The exploring electrode was mounted with an angle 50° on a micromanipulator (Brinkmann, model MH) which allowed vertical movements of the electrode with an accuracy of 0.01 mm. The indifferent electrode was placed at the periphery of the incubation bath, 2.0 cm away from the exploring electrode.

The isolated rat phrenic nerve-hemidiaphragm preparations were indirectly stimulated by an extra-cellular bipolar platinum wire placed in polyethylene tubing. The nerves were stimulated initially with supramaximal 0.2 m/sec single square pulses delivered through a stimulus isolator unit (Nihon Kohden, Model SS201 J).

RMP, AP and MEPPs were recorded after a stabilization period of about 30 minutes. Initially potentials are recorded from control and magnetically exposed animals. Ketamine was added to the bath solution successively in increasing concentrations and after a period of stabilization for 20 to 30 minutes for each concentration, potentials were again recorded. pH of all ketamine (Ketalar, Parke and Davis and Co.) concentrations dissolved in Krebs solution were adjusted to pH 7.4.

RESULTS AND DISCUSSION

The values RMP, AP and MEPP of the control and subject groups are demonstrated in Tables 1 and 2. The values determined after electromagnetic field exposure are shown in Table 1. Table 2 includes the values taken from exposed preparations, by adding Ketamine into the perfusion solution.

Statistical evaluation was performed by students' t test and variance analysis. [CSS (Complete Statistical System), ANOVA 2 way repeated measurement test]. All values were expressed as the mean ± SD.

Resting membrane and action potentials

RMP and AP values recorded from the subject

group were very significantly different ($p<0.001$) from those of the control group (Table 1).

The most important determinants of the RMP are the changes in K ions' conductance, and ratios and intracellular and extra-cellular K ion concentrations ($[K^+]_o/[K^+]i/$) (9,10). Gmitrove *et. al.* (11) observed that extra-cellular potassium ion concentration ($[K^+]_o$) increases and sodium ion concentration ($[Na^+]_o$) decreases in rats exposed to magnetic field. It is now a proven fact that RMP decreases as $[K^+]_o$ increases (12,13). We therefore conclude that decreased RMP value in magnetic field exposed rats observed in the experiments reported here may be due to the increment in $[K^+]_o$ (Table 1).

When RMP approach the critical membrane potential, the excitability increases; experiments performed in vivo on humans and rats, that were exposed to magnetic field, it was demonstrated that the excitability of peripheral nerves increases (5,6). The magnetic field affects the conductance of the ions channels and leads to changes in ion channel conformation and kinetics (14-17).

Table 1: RMP, AP and MEPP's of the rat hemidiaphragm muscles of control and subject groups.

Parameters		Control group (n=200)	Magnetic Field Exposed Group (n=102)	Student t-test
Membrane Potential (mV)		-76.5±2.4 Min=70 Max=83	-72.8±1.8 Min=70 Max=76	P<0.001
Action Potential	Amplitude (mV)	100±3.0 Min=91 Max=110	90.3±2.3 Min=84 Max=96	P<0.001
	Latent period (ms)	0.67±0.1 Min=0.5 Max=1.0	1.13±0.2 Min=0.9 Max=2.0	P<0.001
	Overshoot (mV)	23.5±2.1 Min=19 Max=28	17.2±1.9 Min=14 Max=21	P<0.001
Miniature Endplate Potential	Amplitude (mV)	0.62±0.1 n=18 Min=0.4 Max=1	0.55±0.08 n=13 Min=0.5 Max=0.7	P>0.05
	Frequency (Hz)	0.56±0.02 n=18 Min=0.55 Max=0.58	0.56±0.02 n=13 Min=0.55 Max=0.62	P>0.05

Extension of the latent period

A statistically significant ($p<0.001$) difference was determined between the mean values of latent periods recorded from the control and subject groups. The mean latent period measured from the subject group was found to increase 69%, compared to that of the control group (Table 1). The reasons leading to extension of latent period may be as follows:

a- Magnetic field causes the expansion of synaptic gap in a magnitude of 15% (18). This fact leads the Acetylcholine molecules to diffuse in a longer time; therefore the number of molecules reaching the postsynaptic membrane is reduced (19).

b- Magnetic field exposure decreases the survival of the ligand-receptor complex, and changes the distribution of the membrane receptors (3); these facts together lead to the decrement of Ach receptors in the postsynaptic membrane.

Miniature end-plate potential (MEPP)

We conclude that the decreased mean MEPP amplitude in the subject group may be due to the extracellular $[Na^+]_o$ concentration.

MEPP frequency is dependent on the pre-synaptic membrane (20, 21), and it is primarily effected by the oscillating magnetic field, rather than the static magnetic field (21). Magnetic field exposure decreases the release of Ach and therefore MEPP frequency, calcium transport (7).

Because we could not record MEPPs continuously, changes in MEPP frequency could not be determined in this study (18).

Ketamine

Ketamine leads to depolarization of the skeletal muscle membrane in a dose-dependent manner

(22,23). Ketamine effects the potassium channels of the membrane in resting conditions, and the sodium channels in the active state (24). In order to determine whether magnetic field potentiates the effects of ketamine on these channels, we have explored the combined effects of ketamine and magnetic field.

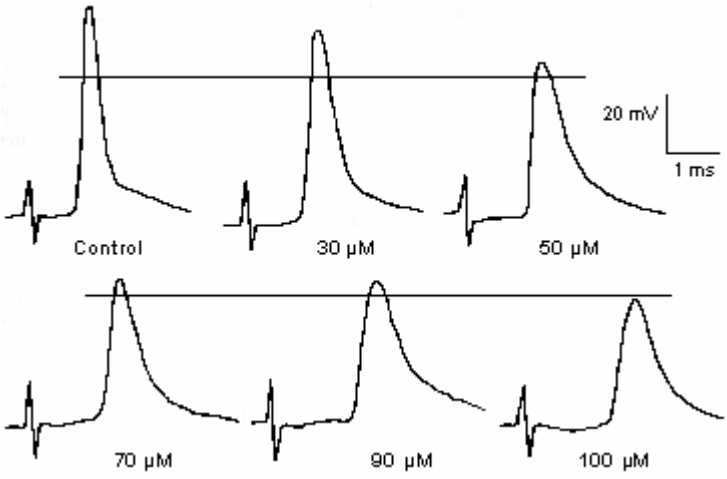
The effects of ketamine on the magnetic field exposed, and non-exposed preparations are shown on Table 2.

In the control group, RMP decreases very significantly ($p<0.000$) in relation to the dose of ketamine; in other words, ketamine causes membrane depolarization (Table 2). Ketamine leads to depolarization by decreasing potassium conductance (23-25). Our results indicate that ketamine potentiates the effect of magnetic fields. Ketamine and magnetic field exposure have similar effects on the skeletal muscle membrane; their combined effect leads the action potential amplitude to decrease and its duration to increase in a much greater magnitude (Table 2, Figure 2).

Table 2: Effects of ketamine on RMP and AP, recorded from hemidiaphragm nerve-muscle preparations of control and magnetic field exposed groups. AP' were not observed at 120 μM of ketamine concentration (n=number of recordings).

GROUPS	BATH KETAMINE CONCENTRATION (μM)	MEMBRANE POTENTIAL (mV)	PARAMETERS OF ACTION POTENTIAL (AP)		
			AMPLITUDE (mV)	LATENT (ms)	OVERSHOOT (mV)
CONTROL GROUP	0 μM KETAMINE n=200	-76.5±2.4	100.1±2.9	0.7±0.1	23.5±2.1
	30 μM Ketamine n=89	-74.7±1.5	92.4±2.5	0.8±0.2	17.6±1.7
	50 μM Ketamine n=90	-74.4±1.9	88.2±2.6	0.9±0.2	15.4±2.8
	70 μM Ketamine n=94	-74.0±2.0	85.1±2.8	1.0±0.4	11.1±3.0
	90 μM Ketamine n=94	-73.6±1.7	77.6±4.5	1.0±0.2	4.1±4.0
	100 μM Ketamine n=97	-72.4±1.8	72.0±2.9	1.2±0.4	-0.5±2.3
	120 μM Ketamine n=90	-70.8±1.3	AP Not observed	AP Not observed	AP Not observed
MAGNETIC FIELD EXPOSED GROUP	0 μM KETAMINE n=102	-72.8±1.8	90.3±2.3	1.1±0.2	17.2±1.8
	30 μM Ketamine n=45	-72.8±1.8	89.7±2.1	1.2±0.3	16.7±1.6
	50 μM Ketamine n=45	72.3±1.4	87.5±2.0	1.4±0.2	14.0±1.3
	70 μM Ketamine n=45	-71.8±1.1	80.7±2.1	1.7±0.2	8.9±1.8
	90 μM Ketamine n=45	-71.4±1.1	73.4±1.6	1.9±0.2	1.9±1.3
	100 μM Ketamine n=45	-70.9±0.9	70.2±1.2	2.1±0.2	-0.8±1.2
	120 μM Ketamine n=45	-69.8±1.0	AP Not observed	AP Not observed	AP Not observed

Figure 2: Values of AP parameters recorded from magnetic field exposed preparations. Changes in relation to concentration of ketamine.



It may be concluded that magnetic field causes RMP and AP amplitude to decrease; latent period and AP duration to increase, by decreasing the conductance of sodium and potassium ions together.

REFERENCES

1. Gould JL : Magnetic field sensitivity in animals. Ann Rev Physiol, 46:585-598, 1984.

2. Tenfordets : Biological interactions of extremely-low-frequency electric and magnetic fields. Bioelectrochemistry and Bioenergetics, 25:1-17, 1991.

3. Adey WR : Tissue interactions with non-ionizing electromagnetic fields. Physiol Rev, 61:435-514, 1981.

4. Rosen AD, Lubowsky J : Magnetic field influence on central nervous system function. Experimental Neurology, 95:679-687, 1987.

5. Hong CZ : Static magnetic field influence on human nerve function. Arch Phys Med Rehabil (United States), 63:162-164, 1987.

6. Hong CZ, Harmon D, Yu J : Static magnetic field influence on rat tail nerve function. Arch Phys Med Rehabil, 67:746-749, 1986.

7. Rosen AD : Magnetic field influence on acetylcholine release at the neuromuscular junction. Am J Physiol, 262:C1418-C1422, 1992.

8. Perry WLM : Pharmacological Experiments on Isolated preparations: By Staff of the Department of Pharmacology University of Edinburgh. Churchill, Livingstone, 1970.

9. Patton HD, Fuchs AF, Hille B, Scher AM, Steiner R : Textbook of physiology. Excitable cells and neurophysiology. WB Saunders Company, 21st Edition, Vol 1, 1989.

10. Robert P : Bioelectric phenomena. New York, Mc Graw-Hill, 1969.

11. Gmitrova A, Ivanco I, Gmitrov J, Murin M : Biological Effect of Magnetic field on Laboratory Animals. Bioelectricity, 7:123-124, 1988.

12. Günay I, Itegin M : Electrical Parameters of Frog Skeletal Muscle. The I. International Biophysics Congress and Biotechnology at GAP. Diyarbakir, Türkiye, 1991.

13. Hodgkin AL : The ionic basis of nervous conduction. Science, 145:3637, 1964.

14. McLeod BR, Smith SD, Cooksey KE, Liboff AR : Ion cyclotron resonance frequencies enhance Ca mobility in diatoms. J Bioelectricity, 6:1-12,1987.

15. McLeod BR, Liboff AR : Dynamic characteristics of membrane ions in multi-field configurations of low-frequency electromagnetic radiation. Bioelectromagnetics, 7:177-189, 1986.

16. Liboff AR, McLeod BR : Kinetics of channelized membrane ions in magnetic fields. Bioelectromagnetics, 9:39-51,1988.

17. Smith SD, McLeod BR, Liboff AR, Cooksey KE : Calcium cyclotron resonance and diatom mobility. Bioelectromagnetics, 8:215-227,1987.

18. Itegin M : Effect of strong static magnetic field on Bioelectricity, biomechanics, Histologic and Atpase enzyme levels of rat hemidiaphragm nerve-muscle preparate. Doctoral Thesis, Adana, 1992.

19. Ganong WF : Review of Medical Physiology. Ed by

Appleton and Lange, Publ Div Prentice Hall, 15th edition, California, pp 11-12, 1991.

20. Gundersen R, Greenebaum B, Schaller M : Intracellular recording during magnetic field application to monitor neurotransmitter release events: Methods and preliminary results. Bioelectromagnetics, 7:271-281, 1986.

21. Katz B : Nerve, muscle and synapse. McGraw-Hill Book Company, 1966.

22. Wachtel RE : Ketamine decreases the open time of single-channel currents activated by acetylcholine. Anesthesiology, 68:563-570, 1988.

23. Gallagher JP, Higashi NDH, NIishi S : Actions of Ketamine on Synaptic Transmission in Frog Sympathetic Ganglia. Neuropharmacology, 15:139-143, 1976.

24. Maleque MA, Warnick JE, Aalbuwuerwue EX : The mechanism and site of action of ketamine on skeletal muscle. J Pharmacol Exp Therap, 219:3, 1981.

25. Lee JH, Frank GB : Opioid effects of racemic ketamine on the excitability of sciatic nerve and skeletal muscle fibers of the frog. Japan J Pharmacol, 51:321-327, 1989.

Correspondence:
Mustafa Itegin
Department of Physiology,
Faculty of Medicine,
Çukurova Univ, 01330 Balcali,
Adana, TÜRKIYE.